

## Contamination of *Bacillus cereus* in Foods Sold at Ramadan Bazaar in Kuching, Sarawak

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### ABSTRACT

Every year there are reports of food poisoning involving food sold at the Ramadan bazaar and one of the causes of food poisoning is *Bacillus cereus*. This study aimed to determine the contamination level of *B. cereus* and the antimicrobial susceptibility profile of the isolates in food sold at Ramadan bazaar in Kuching, Sarawak. A total of 155 samples from five food categories were collected from six Ramadan bazaars in three locations around Kuching. Overall, 32 (21%) food samples were found positive for *B. cereus*. Cooked rice showed the least satisfactory levels of *B. cereus* among the food categories and nasi lemak was the type of food that highly contaminated with *B. cereus*. Antibiotic susceptibility assessment showed that *B. cereus* isolates were highly resistant to ampicillin, amoxicillin/clavulanic, penicillin and cefotaxime in this study. The findings of this study showed that *B. cereus* has the potential to cause food poisoning to Ramadan bazaar consumers due to the high mean count in some types of food. Therefore, this study can be used as basic data for *B. cereus* control measures in food sold at the Ramadan bazaar.

**Keywords:** Antimicrobial susceptibility profile, *Bacillus cereus*, microbiological quality, Ramadan bazaar, Sarawak

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### INTRODUCTION

Food sold at Ramadan bazaar is popular during Ramadan month in Malaysia. A variety of foods from main dishes to desserts are sold at the Ramadan bazaar. Ready-to-eat (RTE) foods such as cooked rice, cooked meat or fish, cooked vegetables, beverages, sweet cakes and noodles are widely sold at Ramadan bazaar. Although Ramadan bazaar food is convenient for consumers during the month of Ramadan, there have been several cases of food poisoning involving food sold at Ramadan bazaar. For example, a total of 93 cases of food poisoning have been recorded in Terengganu, Malaysia since the first day of fasting on April 3, 2022 caused by food bought at Ramadan bazaars and roadside vendors. In the same report, 222 notices in accordance with the Food Hygiene Regulations 2009 for various offenses during the inspection of food handlers at Ramadan bazaar were also issued. Most of the offenses involved

not attending the food handler course and failing to meet the dress code (Bakar, 2022). In another report, Ramadan bazaar food samples were also analysed for the level of microbiological contamination and found that microorganisms such as *Staphylococcus aureus* in duck egg curry and *Salmonella* spp. in chicken-based food and cendol were detected in Kelantan, Malaysia (Idris, 2022).

Food-borne illness is defined as any form of an unhealthy condition suffered by the patient after the consumption of food contaminated with pathogens, viruses and parasites (Rusnan *et al.*, 2020). *Bacillus cereus* has been recognized as one of the causes of food-borne illness for many years and estimated to be responsible for 1.4% to 12% of all food poisoning outbreaks worldwide (Dietrich *et al.*, 2021). *Bacillus cereus* is a large, Gram-positive, motile, aerobic-to-facultative, spore-forming rod and has the ability to produce toxins in a variety of foods (Tewari & Abdullah,

2015). *Bacillus cereus* may lead to two types of food poisoning, the emetic and diarrheal syndromes. The diarrheal form of food poisoning is due to combination of various toxins and produced by vegetative growth of the bacteria in small intestine. While, the emetic syndrome is due to small molecular weight toxin, heat and pH stable, which is cereulide produced in the food (Bilung *et al.*, 2018a; Gdoura-Ben Amor *et al.*, 2018; Elise *et al.*, 2019). Emetic syndrome is usually characterised by nausea and vomiting, which appears in a few hours after consuming contaminated food (Dietrich *et al.*, 2021).

*Bacillus cereus* is commonly found in rice and rice-based foods and has been known as the normal microflora of rice grains (Bilung *et al.*, 2018; Rusnan *et al.*, 2020). *Bacillus cereus* also can be found in many types of food such as milk product, meat, vegetables and cereal products (Gao *et al.*, 2018; Gdoura-Ben Amor *et al.*, 2018; Fiedler *et al.*, 2019; Saba *et al.*, 2019; Yu *et al.*, 2020). There are several local studies on *B. cereus* occurrence in food such as RTE cooked rice from cafeteria, restaurant and food courts in Penang (Navaneethan & Effarizah, 2021), Ultra High Temperature (UHT) chocolate milk from milk manufacturer (Ubong *et al.*, 2020) and meat curry from restaurant in Selangor and Kuala Lumpur (Msarah *et al.*, 2020). Besides, some other studies reported that *B. cereus* found in foods were resistant against ampicillin, penicillin, amoxicillin/clavulanic and tetracycline (Gao *et al.*, 2018; Fiedler *et al.*, 2019; Park *et al.*, 2020; Yu *et al.*, 2020). Thus, the purpose of this study was to determine the level of *B. cereus* contamination from different types of foods collected at Ramadan bazaar in Kuching, Sarawak and to evaluate the antimicrobial susceptibility profile of isolates in this study.

## MATERIALS AND METHODS

### Sample Collection

For the purpose of this study, 256 Ramadan bazaar's stalls were selected in which the sample size required was determined using a Raosoft sample size calculator with the confidence level

at 95% and the margin of errors at 5%, respectively (Mohd Asmawi *et al.*, 2018). Therefore, the actual sample size was 154. However, a total of 155 samples from different food categories were obtained from six Ramadan bazaar in three locations (Kuching North, Kuching South, Padawan) in Kuching, Sarawak. Foods that were at high risk of food contamination such as cooked meat/fish (24 samples), kuih-muih (47 samples), noodles (33 samples), cooked rice (31 samples) and cooked vegetables (20 samples) were sampled in this study.

### Determination of *Bacillus cereus*

*Bacillus cereus* were analysed by using ISO 7932:2004 (E) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *B. cereus* - Colony-count technique at 30 °C. *Bacillus cereus* ATCC 11778 was used as a reference culture to conformed the performance of medium used in enumeration. *Bacillus cereus* contamination levels were compared to the standard guidelines according to Food Standards Australia New Zealand (FSANZ) Compendium of Microbiological Criteria for Food (2022).

### Antibiotic Susceptibility Test

Antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method recommended by Clinical and Laboratory Standards Institute (CLSI, 2020) using Mueller-Hinton agar. The antibiotic discs tested for *B. cereus* were ampicillin (10 µg), amoxicillin/clavulanic (20/10 µg), amikacin (30 µg), chloramphenicol (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), penicillin, rifampicin (5 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg) and tobramycin (10 µg). The antimicrobial agents tested in this study were selected according to Bilung *et al.* (2018b), Gdoura-Ben Amor *et al.* (2018), Fiedler *et al.* (2019) and Park *et al.* (2020) as no *Bacillus* specific criteria for disc diffusion antibiotic-resistance assays have been defined either by European Committee on Antimicrobial

Susceptibility Testing (EUCAST) or CLSI. The zone of inhibition of growth around each disc was then measured in millimeters and zone diameters interpreted in accordance with standards as sensitive, intermediate and resistant (CLSI, 2020; EUCAST, 2020). Teicoplanin, ampicillin and kanamycin were measured and interpreted referring to the zone diameter interpretive criteria of *S. aureus* in CLSI, 2010 (Gao *et al.*, 2018). Multiple antibiotic resistances (MAR) index for each resistant profile was determined according to Zulfakar *et al.* (2021).

### Statistical Analysis

All statistical analyses were performed using the IBM Statistical Packages for Social Science, SPSS Version 26. The frequencies of positive samples and descriptive statistics such as mean; standard deviation were obtained. A one-way ANOVA was conducted to compare levels of bacterial contamination between locations and food categories. Statistically significant differences were performed at a 5% significance level.

## RESULTS

### *Bacillus cereus* in Ramadan bazaar foods

Table 1 represents *B. cereus* counts of different varieties of foods sold at Ramadan bazaar in three locations. Only 21% (32/155) of the total samples were contaminated with *B. cereus*.

*Bacillus cereus* were distributed at all locations and in three types of food categories. Contamination of *B. cereus* at Ramadan bazaar in Kuching North showed the highest mean value of  $3.769 \pm 0.613$  Log CFU/g. Meanwhile, cooked rice showed the most contaminated food category followed by kuih-muih and noodles. *Bacillus cereus* in cooked rice demonstrated the highest mean value of  $3.505 \pm 0.819$  Log CFU/g. The results revealed the significant differences of *B. cereus* between three locations ( $p < 0.05$ ) but no significant differences among food categories ( $p > 0.05$ ). No *B. cereus* were found in cooked meat/fish and cooked vegetables in this study. Out of five food categories, three of them were found to be contaminated with *B. cereus*; cooked rice (14), noodles (7) and kuih-muih (11) as shown in Figure 1. Nasi lemak and mee jawa showed the most contaminated food in cooked rice and noodles category, respectively. Meanwhile, in the category of kuih-muih, buah melaka and roti jala exhibited a greater prevalence of food contamination.

### Comparison of *Bacillus cereus* Contamination Level with Microbiology Standard

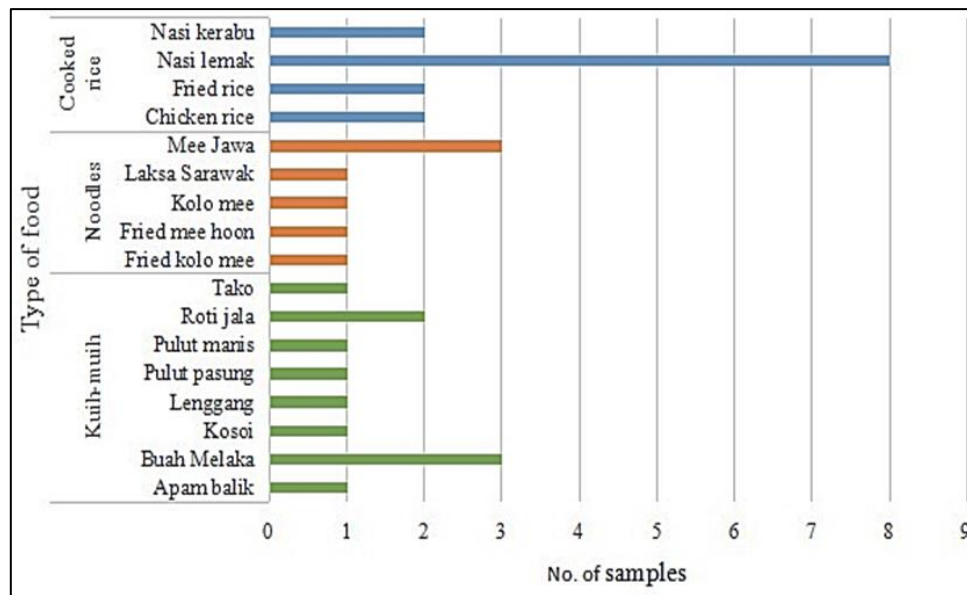
Contamination of *B. cereus* was detected in 21% of all samples tested. In Malaysia, based on the Food Regulations 1985 and the Food Act 1983, there is no specific microbiological standard for *B. cereus*.

**Table 1.** *Bacillus cereus* count in Ramadan bazaar foods

Location	N <sup>1</sup>	Mean <sup>2</sup>	± SD	p-value
Kuching North	16	3.769	0.613	0.002
Kuching South	1	2.000	.	
Padawan	15	3.087	0.583	
Total	32	3.394	0.719	
Food Category				
Noodles	7	3.276	0.837	0.750
Kuih-muih	11	3.329	0.526	
Cooked rice	14	3.505	0.819	
Cooked meat/fish	0	.	.	
Cooked vegetables	0	.	.	
Total	32	3.394	0.719	

<sup>1</sup>Number of positive samples

<sup>2</sup>Mean bacterial counts expressed in Log CFU/g, SD: standard deviation



**Figure 1.** Type of food samples contaminated with *Bacillus cereus*

According to the standard guidelines (FSANZ Compendium of Microbiological Criteria for Food, 2022), *B. cereus* is deemed unsatisfactory if its presence falls within the range of 3 Log CFU/g to  $\leq 5$  Log CFU/g, and it is considered potentially harmful if the contamination level exceeds 5 Log CFU/g. Table 2 shows the

contamination level of *B. cereus* in food categories. Based on the table, 79% of the total samples was at a satisfactory level, followed by 5% at marginal level and 16% was at unsatisfactory level of contamination. Cooked rice showed as the most unsatisfactory level (35%) among the food categories.

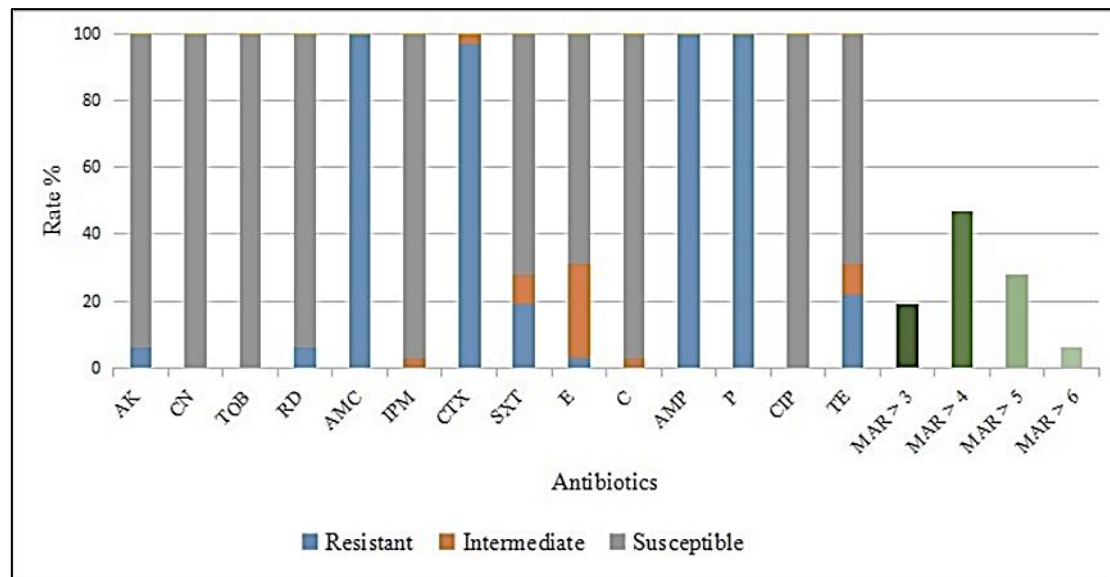
**Table 2.** Contamination level of *Bacillus cereus* in Ramadan bazaar foods

Food Category	Contamination rate (%)	No. of satisfactory samples (%)	No. of marginal samples (%)	No. of unsatisfactory samples (%)
		< 2 Log CFU/g	2 Log CFU/g – < 3 Log CFU/g	3 Log CFU/g – $\leq 5$ Log CFU/g
Noodles	7/33 (21)	26/33 (79)	2/33 (6)	5/33 (15)
Kuih-muih	11/47 (23)	36/47 (72)	2/47 (4)	9/47 (19)
Cooked rice	14/31 (45)	6/31 (19)	3/31 (10)	11/31 (35)
Cooked meat/fish	0/24 (0)	24/24 (100)	0/24 (0)	0/24 (0)
Cooked vegetable	0/20 (0)	20/20 (100)	0/20 (0)	0/20 (0)
Total	32/155 (21)	123/155 (79)	7/155 (5)	25/155 (16)

### Antimicrobial Susceptibility of *Bacillus cereus* Isolates

In this study, 32 isolates of *B. cereus* were tested for their sensitivity profile against 15 selected antibiotics. As shown in Figure 2, 100% of the isolates were sensitive against ciprofloxacin, gentamicin and tobramycin. Followed by chloramphenicol (97%) and imipenem (97%). The highest rate of resistance was to ampicillin (100%), amoxicillin/ clavulanic (100%), penicillin (100%) and cefotaxime (97%). In

addition, 22% of the total isolates were resistant to tetracycline followed by trimethoprim-sulfamethoxazole (19%). MAR index of *B. cereus* isolates in this study ranged between 0.27 - 0.47. According to the definition of MAR (Gao et al., 2018), all isolates in this study were defined as MAR strains. Approximately 28% of the isolates exhibited resistance to five or more types of antibiotics, while a smaller proportion of around 6% showed resistance to more than six different types of antibiotics.



**Figure 2.** Antimicrobial susceptibility rate and *Multiple Antibiotic Resistance* profile. AK- Amikacin, CN- Gentamicin, TOB- Tobramycin, RD- Rifampicin, AMC- Amoxicillin/clavulanic, IPM- Imipenem, CTX- Cefotaxime, SXT-Trimethoprim-sulfamethoxazole, E- Erythromycin, C- Chloramphenicol, AMP- Ampicillin, P- Penicillin, CIP- Ciprofloxacin, TE- Tetracycline

## DISCUSSION

In this study, 32 (21%) of the 155 food samples were positive for *B. cereus* indicating foods sold at Ramadan bazaar have a potential risk to cause food poisoning to the consumers. A total of 25 (16%) samples exceeded the standards set by the FSANZ Compendium of Microbiological Criteria for Food and can potentially cause illness if ingested in high concentrations (Rusnan *et al.*, 2020). Various types of cooked rice, kuih-muih and noodles are among the potential sources of *B. cereus* in the Ramadan bazaar. In Malaysia, rice is the staple food and a study conducted by Bilung *et al.* (2018a) showed a high concentration of *B. cereus* in all unhusked rice samples in Sarawak, Malaysia. The findings indicate the potential for processed rice to be contaminated with *B. cereus*, thus increasing the risk to humans if control measures are not taken before consumption.

In the present study, *B. cereus* were found in nasi lemak, nasi kerabu, fried rice and chicken rice. This result is in line with several studies that found cooked rice contaminated with *B. cereus*. A study done by Saba *et al.* (2019) reported street food fried rice recorded the highest number of unsatisfactory samples of *B. cereus*. Navaneethan and Effarizah (2021) revealed the occurrence of *B. cereus* in 34 of RTE cooked rice samples in the range of 3.51 – 5.95 Log CFU/g.

Another study in India and China also reported the presence of *B. cereus* in cooked rice (Vedesh & Neel, 2017; Yu *et al.*, 2020). These indicate contamination and presence of *B. cereus* in cooked rice are very common. The results of previous studies not only reported that cooked rice was contaminated with *B. cereus* but there were also other foods such as cooked chicken meat, pasteurized milk, pastry products, noodles, seafood products and canned products (Gao *et al.*, 2018; Gdoura-Ben Amor *et al.*, 2018; Yu *et al.*, 2020).

In addition, *B. cereus* was found in spices and herbs (Fogele *et al.*, 2017). Due to the addition of spices and herbs in food preparation such as meat and rice, it may introduce the contamination of *B. cereus* in the food. In Thailand, *B. cereus* was found in traditional foods such as Thai pandan custard and the source of the contaminant appears to be from pandan leaves (Rusnan *et al.*, 2020). As in Thailand, pandan leaves are also frequently used for cooking in Malaysia such as in nasi lemak and buah melaka which are the most contaminated food in this study. The contamination was believed to occur due to cross-contamination of raw food materials and poor sanitation in food preparation. In addition, an adequate temperature range should be followed during cooking to make the food safer (Saba *et al.*, 2019). Insufficient heat and leaving the foods at

room temperature allows *B. cereus* to further multiply (Rusnan *et al.*, 2020). According to Tewari and Abdullah (2015), rapid cooling and reheating of properly cooked food is very important if the food is not consumed immediately and long-term storage must be at a temperature below 8 °C. Since the foods at the Ramadan bazaar have been prepared earlier by the food handlers, it is suggested for consumers to reheat food such as Laksa Sarawak, mee jawa, chicken rice and to refrigerate traditional Malay kuih such as sweet cakes.

In this study, all isolates were resistant towards  $\beta$ -lactam antibiotics, ampicillin (100%) and penicillin (100%), which is in good agreement with previous studies (Khasnabis *et al.*, 2017; Bilung *et al.*, 2018b; Gao *et al.*, 2018; Yu *et al.*, 2020). High resistance towards ampicillin and penicillin is expected due to the fact that *B. cereus* is genetically resistant to all  $\beta$ -lactam except carbapenems (Ikeda *et al.*, 2015; Bilung *et al.*, 2018b). All isolates were also fully resistant to  $\beta$ -lactam/ $\beta$ -lactamase inhibitor combinations, amoxicillin/clavulanic (100%), which is similar with studies by Yu *et al.* (2020) and Khasnabis *et al.* (2017). The high resistance of isolates to ampicillin, penicillin and amoxicillin/clavulanic may be due to the widespread use of these antibiotics in the clinical setting (Jamaluddin *et al.*, 2021; Zulfakar *et al.*, 2021). In addition, 97% of the isolates were resistant to cefotaxime and consistent with the study by Fiedler *et al.* (2019) and Park *et al.* (2020) who reported high resistance rates in vegetables in Germany and Korea, respectively. It is interesting to note that isolates from food showed high resistance to cefotaxime compared to clinical samples (Ikeda *et al.*, 2015). Moreover, some of the *B. cereus* isolates displayed multiple-drug resistance profiles with 28% of isolates showed resistance to five or more types of antibiotics that need more attention and may pose a potential health risk to consumers (Khadka *et al.*, 2018). On the other hand, all the 32 *B. cereus* isolates were susceptible to ciprofloxacin, gentamicin and tobramycin. Similar to this, Gdoura-Ben Amor *et al.* (2018) received 100% sensitivity to ciprofloxacin and gentamicin in samples from food stuffs in Tunisia. Most of the isolates in this study were also sensitive to chloramphenicol (97%), which is significantly higher than the sensitivity rate reported by Bilung *et al.* (2018b) from a sago processing plant (48%). About 97%

of the isolates were sensitive to imipenem and since imipenem is one of the carbapenems antibiotics, further study is needed to determine whether carbapenems can be one of the options for the empirical treatment of *B. cereus* as suggested by Ikeda *et al.* (2015). The antimicrobial susceptibility profile of *B. cereus* in present study differs from previous studies probably due to the different isolated sources, varies in the concentration of antimicrobial agents used and the widespread use of antibiotics in the environment (Bilung *et al.*, 2018b).

## CONCLUSION

In conclusion, *B. cereus* is the potential contaminating agent of the Ramadan bazaar food, particularly in cooked rice, kuih-muih and noodles. Some of *B. cereus* isolates in this study exceeds the acceptable level and displayed multiple-drug resistance profiles. The findings represent the first research on microbiological quality and safety that specifically covers Ramadan bazaar food in Kuching, Sarawak and can be helpful for policy makers to develop more intervention strategies to prevent this issue from recurring. However, this study has limitations as no *Bacillus* specific criteria for disc diffusion antibiotic resistance testing have been defined by either EUCAST or CSLI and it is hoped that some *B. cereus* antimicrobial breakpoints will be established in the future. Since the results of the study demonstrated the quality and safety of the food sold in Ramadan is quite unsatisfactory, thus it is suggested a comprehensive training for food handlers in the Ramadan bazaar, particularly on proper food handling as well as food safety awareness for consumers.

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